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The conserved type of *Lichen fuscatus* [≡ *Acarospora fuscata*]

Kerry Knudsen[1*], Jiří Malíček[2], Jana Kocourková[1]

[1] *Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Prague, Kamýcká 129, Praha 6 - Suchdol, CZ–165 00, Czech Republic*

[2] *The Czech Academy of Sciences, Institute of Botany, Zámek 1, CZ-252 43 Průhonice, Czech Republic*

**Correspondence TO: knudsen@fzp.czu.cz*

Abstract—The conserved type of *Lichen fuscatus,* the basionym of *Acarospora fuscata,* is described, and two genes, ITS and mrSSU, are made available through GenBank for further phylogenetic research.

Key words—*Acarospora gallica*, *A. variegata*, nomenclature, taxonomy

Introduction

Acarospora fuscata is the most common species of the genus in Europe (Magnusson 1929). In crustose lichen communities on siliceous rock, its pale brown color is distinctive, and it is often collected. But despite *A. fuscata* being common, it did not have a designated type or legitimate name (Arcadia & al. 2015). Because the taxon is common and the name *A. fuscata* is in wide use, a formal proposal was made to conserve the name *Lichen fuscatus* Schrad. (Arcadia & al. 2015) and has been accepted (May 2017). A modern conserved type that could be sequenced was designated that matched the species concept of A.H. Magnusson, who did the most extensive study of the species in Europe (Magnusson 1929). The taxon's most typical phenotype was collected in central Europe to serve as conserved type. In this paper, we present a description of the conserved holotype and isotypes and designate GenBank sequences to aid in the identification and phylogenetic study of *A. fuscata* and other *Acarospora* taxa containing gyrophoric acid that have been confused with it.

Material & methods

The description is based only on the conserved holotype and isotypes. Specimens were studied with standard microscopy. Measurements were made in water. Undiluted fresh Merck Lugol's (IKI) was used to test amyloid reactions of thin squashed sections of apothecia following Knudsen & Kocourková (2018). Ascus structure was studied with IKI with or without pretreatment with KOH (Hafellner 1993). Thin-layer chromatography (TLC) was used to verify the presence of secondary metabolites (Orange & al. 2001).

DNA was extracted from the holotype (Kocourková 8499, S) using the Invisorb Spin Plant Mini Kit. The fungal ITS rDNA and mitochondrial SSU were amplified with the following primers: ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990), and mrSSU1 and mrSSU3R (Zoller & al. 1999). PCR reactions of nrITS and mtSSU were prepared for a 20 µl final volume containing 14 µl double-distilled water, 4 µl MyTaq polymerase reaction buffer, 0.2 µl MyTaq DNA polymerase, 0.4 µl of each of the 25 mM primers, and 1 µl of the sample. Amplifications of both loci comprised an initial 1 min denaturation at 95 °C; 35 cycles of 1 min at 95 °C, 1 min at 56 °C, 1 min at 72 °C; and a final 7 min extension at 72 °C. The PCR products were visualized on a 0.8% agarose gel and cleaned with ExoSAP-IT, according to Thermo Fisher Scientific protocols. Both newly produced sequences, ITS (MK178225) and mrSSU (MK178225), were checked in BioEdit 7.2.5 (Hall 1999).

Macrophotographs were taken with an Olympus DP74 camera mounted on an Olympus SZX 7 stereomicroscope equipped with PRO-SZM1-Focus Drive Motorization for stacking pictures and stacked using the Olympus DeepFocus 3.4 module. The figure plates were processed with QuickPhoto Camera 3.2 software fitted with Promicra Publisher Module and eventually refined with Adobe Photoshop CS4 Extended ver. 11.0. The type locality picture was taken with Panasonic DMC LX5.

Taxonomy

Acarospora fuscata (Schrad.) Arnold,
Flora 53 (30–31): 469. 1871 ["1870"]. Fig. 1

≡ *Lichen fuscatus* Schrad., Spic. Fl. Germ.: 83. 16 Mai–5 Jun 1794, nom. cons. prop.

Type: Czech Republic. West Bohemia, Distr. Tachov, between Mezholezy and Racov, Přírodní park Sedmihoří (Natural Park), 49°37′53″N 12°51′41″E, 524 m, granite outcrops in *Pinus sylvestris* forest, 1 Oct. 2014, J. Kocourková 8499 & K. Knudsen (S, **conserved holotype**, designated in Arcadia & al. 2015; B, FH, KRAM, GZU, H, NY, PRA, UCR, UPS, conserved isotypes).

Thallus indeterminate of areoles, often contiguous, occasionally imbricate, covering areas sometimes more than a meter wide, or dispersed, sometimes forming lines along fractures in the rock. Areoles irregular in shape, often lobulate with edges free and often with a black margin, sometimes reduced to lecanorine-like apothecia, broadly attached to the substrate, replicating by division, 0.1–3.0 mm wide, up to 1 mm thick. Upper surface pale brown,

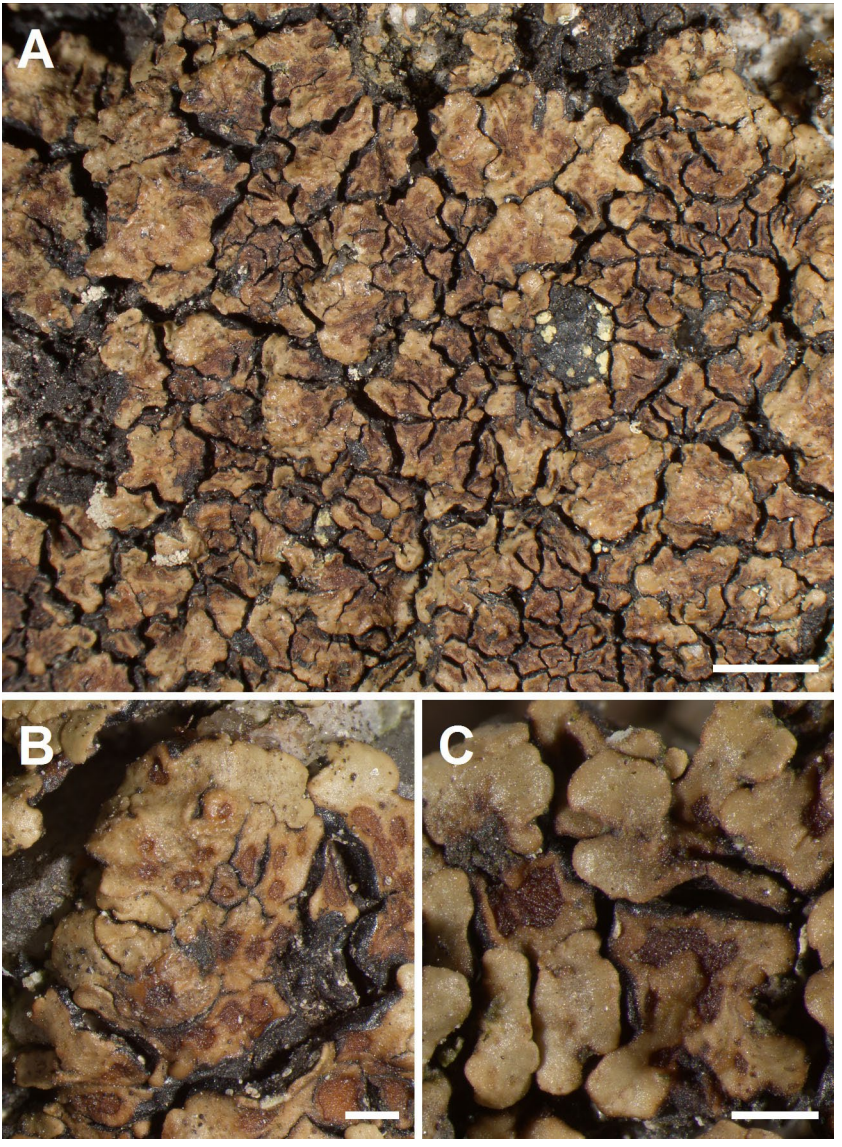

Fig. 1. *Lichen fuscatus* (conserved holotype, J. Kocourková 8499, S): A. Habit of the areolate thallus with apothecia; B. Marginal sublobate areole with sunken apothecia; C. Detail of areoles with apothecia. Scale bars: A = 2 mm; B, C = 500 µm.

epruinose, matte, smooth. Lower surface light or dark brown, corticate under the margins, becoming black through melanization. Epicortex thin (<10 µm

thick) or absent. Cortex 25–50 µm thick, upper layer yellow brown, lower layer hyaline, paraplectenchymatous, with cells 3–5 um diam., perpendicularly arranged and usually distinct, containing crystals visible in polarized light. Algal layer 50–100 µm thick, uninterrupted by hyphal bundles, with cells 5–12 µm diam. Medulla forming a thick mycelial base (gomphate), 0.1–0.8 mm thick, not distinctly stipitate, the outer surface usually carbonized, medullary hyphae intricate, thin-walled, septate, 3–4 µm thick.

Apothecia solitary or up to 10 per areole, immersed, punctiform 0.1–0.3 µm or expanding ≤0.6 mm wide, disc round or irregular, reddish-brown, darker than the thallus, epruinose, smooth or rough. Parathecium 10–20 µm diam., not expanding around the disc. Epihymenium 10–15 µm thick, reddish-yellow, coherent. Hymenium (80–)100–120(–140) µm high, paraphyses (1.0–)1.5–2.0 µm diam. at mid-level, not branching, septate, often with oil drops, with the apices barely expanded or capitate, 3–5 µm diam., hymenial gel IKI+ red or blue turning red (hemiamyloid). Asci 60–90 × 15–18 µm, ascus stain *Acarospora*-type, ascospores 4.0–5.0 × 1.5–2.0 µm, narrowly ellipsoid to ellipsoid. Subhymenium 30–60 µm thick, IKI+ blue (euamyloid). Hypothecium 10–20 µm thick. Pycnidia 100–110 × 60–70 µm, conidiogenous cells ampulliform, conidia 1.0–2.0 × 0.5–1.0 µm. Secondary metabolite: gyrophoric acid in cortex, C+, KC+ pinkish red in thin section. Sequences available in GenBank: ITS (MK178225) and mrSSU (MK178225).

Ecology & Substrate—The conserved type was collected at a low (310 m) elevation in full sunlight in *Pinus sylvestris* forest, where it covered the tops of granite outcrops without other associated lichens (Fig. 2)

Comments—Using our description and simple spot tests many specimens of *A. fuscata* can now be easily identified. Our description based on the conserved type does not differ significantly from the descriptions of Magnusson (1929) or Fletcher & al. (2009), except with respect to hymenium height. In the type the hymenium measures (80–)100–120(–140) µm, varying 60 µm in height depending on apothecial size. Magnusson (1929) gives a height of (70–)85–100 µm, a range of 30 µm. The British flora cites (70–)80–120 µm, a range of 50 µm (Fletcher & al. 2009).

Nonetheless, *Acarospora fuscata* exhibits a wide phenotypic variability in Europe, which led to the description of nine forms and one variety (Magnusson 1929). Now that we have gene sequences available from the conserved type of *A. fuscata,* unusual specimens can be verified with molecular analyses. Additional sequences can also be made from the conserved type or matching specimens, so that researchers can finally

Fig. 2. *Acarospora fuscata* type locality in West Bohemia, Czech Republic

establish if *A. fuscata* is polymorphic or a complex of several semi-cryptic or cryptic taxa. Candidates for a full genomic study can be selected that might reveal whether there is horizontal gene transfer with other *Acarospora* species. In Europe, the current taxonomic circumscriptions of *A. gallica* H. Magn. and *A. variegata* H. Magn. can also be revised. The British submontane taxon "*A. peliscypha*" sensu auct. Brit., included in *A. fuscata,* can be analyzed as well as pruinose specimens (Fletcher & al. 2009). The name *A. fuscata*, like other old European names, was applied to brown C+ red *Acarospora* taxa around the world. Additionally, collections from North America, Australia, or Asia identified as *A. fuscata* can be revised (Knudsen 2007, McCarthy & Elix 2017, Ohmura & Kashiwadani 2018).

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